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RESEARCH ARTICLE

INVESTIGATION FOR CONTAMINATIONS OF RAW MILK ON MILK COLLECTION UNITS IN NEVSEHIR PROVINCE

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ABSTRACT

In this study, it was aimed to investigate Aflatoxin M_1 and heavy metal pollution in raw milk samples taken from milk collection centers operating in Nevsehir province. As a result of the analyzes made on raw milk samples; The amount of Aflatoxin M_1 was determined in the range of 0.013-0.150 ppb, in 7 raw milk samples remained below the measurement limit. A limit of 0.05 ppb has been determined for Aflatoxin M1 in the Turkish Food Codex and the amount of Aflatoxin M_1 was determined above the legal limit in 15% of the raw milk samples taken. As a result of heavy metal analysis with ICP-MS in raw milk samples, Mg 41,96±1,88 ppm; Ca 79,83±4,30 ppm; Mn 0,0115±0,007 ppm; Fe 0,176±0,003 ppm; Ni 0,0283±0,002 ppm; Cu 0,0181±0,003 ppm; Zn 1,56±0,005 ppm; As 0,0046±0,000 ppm; Cd 0,001549±0,002 ppm and Pb 0,00048±0,000 ppm were found, respectively. According to our study results, it was determined that there was no risk human health risk in terms of heavy metal.

Keywords: Raw Milk, Heavy Metal, Aflatoxin M_1, Nevsehir

1. INTRODUCTION

Milk is a vital basic food that can meet the needs of the organism in all mammals due to the wide variety of nutrients it contains [1].

Milk has a very important place in human nutrition. It can threaten human health if it is not stored and processed under minimum technical and hygienic conditions.

According to the Turkish Food Codex Contaminants Regulation; "All kinds of foreign substances, such as animal hair, insect fragments, which are not intentionally added to the food but are found in the food as a result of the production, manufacture, processing, preparation, processing, packaging, packaging, transportation or preservation, including the primary production stage of the food, or environmental contamination is defined as contaminants [2].

If an evaluation is made in terms of raw milk; Veterinary drugs, mycotoxins, pesticides, detergent residues used in cleaning and disinfection, and heavy metals, not naturally present in the composition of milk, but contaminate the milk from various sources, can be given as examples for contaminants [2].

The presence of Aflatoxin M_1 in raw milk is an important element that threatens public health. Among animal products, aflatoxin is mostly found in milk and dairy products. There are two main reasons for aflatoxin contamination of milk and dairy products. The first is that Aflatoxin B_1 and Aflatoxin B~~2~~, which are taken by lactating animals as a result of consuming feeds contaminated with molds, are metabolized in the animal body and passed into milk as Aflatoxin M_1 and Aflatoxin M_2. The second is

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caused by the contamination of aflatoxin-synthesizing molds and the production of aflatoxins during the transportation, processing and storage processes after milking [3].

There is no significant change in the amount of Aflatoxin M_1 during pasteurization of milk or processing into other dairy products, and Aflatoxin M_1 passes into dairy products at various rates [4]. Due to the reasons stated, legal regulations have been made by the countries regarding the maximum amount of Aflatoxin M_1 that can be found in milk and dairy products that are frequently consumed by almost all age groups, especially children.

Rapid developments in industry and technology not only make life easier for people, but also increase production and consumption, disrupt natural balances, destroy resources, and pollute air, water and soil. All living things are adversely affected due to environmental pollution, and exposure of plants and animals to toxic pollutants in various ways indirectly threatens human health. Heavy metals and pesticides can be given as examples of these toxic pollutants that contaminate the food chain with environmental pollution [5].

In terms of mineral matter, milk is an important source of Ca and P; Heavy metal contamination can occur in raw milk from the pastures where the animal is fed, from the feed, from the water it drinks, from the containers used to preserve the milk [5-7]. The main elements in metallic contamination arising from metal containers and operating water used during technological processes or for the preservation of milk and its products; metals such as copper, zinc, iron, tin, lead, arsenic, cadmium [8].

In our country, milk processing facilities mainly supply raw milk to be processed from collection centers. Although there are scientific studies on raw milk produced on farms in our country, it is not sufficient and the necessary precautions cannot be taken effectively.

In this study, it is aimed to evaluate the raw milk in terms of contaminants by investigating the presence of Aflatoxin M_1 and heavy metal pollution in raw milk samples taken from milk collection centers operating in Nevşehir province. Our study will have a unique value with the results obtained in terms of the lack of available scientific data on the examination of raw milk collected in Nevşehir province in terms of contaminants.

2. MATERIALS AND METHOD

2.1. Material

In this study, one approved milk collection center operating in each district of Nevşehir was determined. 7 raw milk samples were used as material.

2.2. Aflatoxin M_1 Analysis

A total of 28 raw milk samples were taken and analyzed for Aflatoxin M_1 analysis in sterile sample containers in accordance with the sampling rules from the milk collection centers determined in quarterly periods between January and December 2021.

In Aflatoxin M1 analysis; 10 ml of each sample was weighed. It was homogenized by adding 80 ml of 35°C water. The obtained homogenates were incubated in a water bath at 35°C for 30 minutes. After homogenization, the samples were centrifuged at 4000 rpm for 15 minutes at room temperature and the dilution process was started. In the dilution process, the extract was filtered through whatman no:4 filter paper. After the filtration process was completed, 50 ml of the extract was taken and the adsorption process was started. The milk sample is passed directly through the immunoaffinity column. Mobile phase content Methanol / Acetonitrile / Water (2:3:5 v/v/v/) was used as the running fluid for HPLC.

Aflatoxin M1 mobile phase prepared in this way was kept in an ultrasonic bath for about 15 minutes. The samples were analyzed by reversed phase liquid chromatography (RP-HPLC) [9]

2.3. Determination of Heavy Metal Analysis

Between January and December 2021, a total of 28 raw milk samples were taken from the same milk collection centers determined in quarterly periods, and heavy metal and mineral substance analyzes were carried out in the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

The samples were dissolved in acidic medium by wet burning (microwave oven) method. The metal (Mg, Ca, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb) contents of the samples were measured with the ICP-MS device using certain standards [10].

3. RESULTS

3.1. Aflatoxin M_1 Results

The maximum value that can be found for Aflatoxin M_1 in raw milk is specified as 0.05 ppb in the Turkish Food Codex Contaminants Regulation [2]. The legal limit for Aflatoxin M_1 in China and the USA is 500 ng/L [11].

The lowest value for Aflatoxin M_1 was 0.013±0.003 ppb in the Ürgüp district during July-September 2021; the highest value was determined as 0.150±0.006 ppb in the Gulsehir district during the October-December period. Aflatoxin M_1 level was below the measurement limit of 0.0088 ppb in 25% of the raw milk samples taken.
In 15% of the raw milk samples taken, the maximum value that can be found for Aflatoxin M_1 in raw milk reported in the Turkish Food Codex Contaminants Regulation and the EU 1881/2006 commission regulation was found to be above 0.05 ppb [2, 12].

Aflatoxin M_1 levels of analyzed raw milk samples are given in Table 1.

Table 1. Aflatoxin M_1 levels (ppb)

Districts	January-March 2021	April-June 2021	July-September 2021	October-December 2021
Acıgöl	**ND**	**0,016**	**ND**	**0,030**
Avanos	**0,026**	**0,037**	**0,020**	**0,016**
Gülşehir	**0,078**	**0,120**	**0,093**	**0,150**
Derinkuyu	**ND**	**0,029**	**0,036**	**0,041**
Hacıbektaş	**ND**	**0,030**	**0,027**	**0,027**
Kozaklı	**ND**	**0,036**	**0,043**	**0,025**
Ürgüp	**ND**	**ND**	**0,013**	**0,034**
NOTE: ND (Not Detectable - Limit of Measurement is below 0.0088 ppb.)				

Statistical evaluation of aflatoxin M_1 analysis results was made using SPSS 15.0 package program. Shapiro-Wilk test was applied to determine whether the obtained findings had a normal distribution, and it was determined that the data did not have a normal distribution. The findings were evaluated by applying the Kruskal Wallis H test to examine whether the difference between the sampled districts was significant. When the aflatoxin M_1 analysis results are evaluated, there is a statistically significant difference between the districts according to the 5% significance level ($p<0,05$). When the aflatoxin M1 analysis results were evaluated, there was no statistically significant difference between collection times.

Aflatoxin M_1 concentration percentages of the analyzed raw milk samples are given in Figure 1.

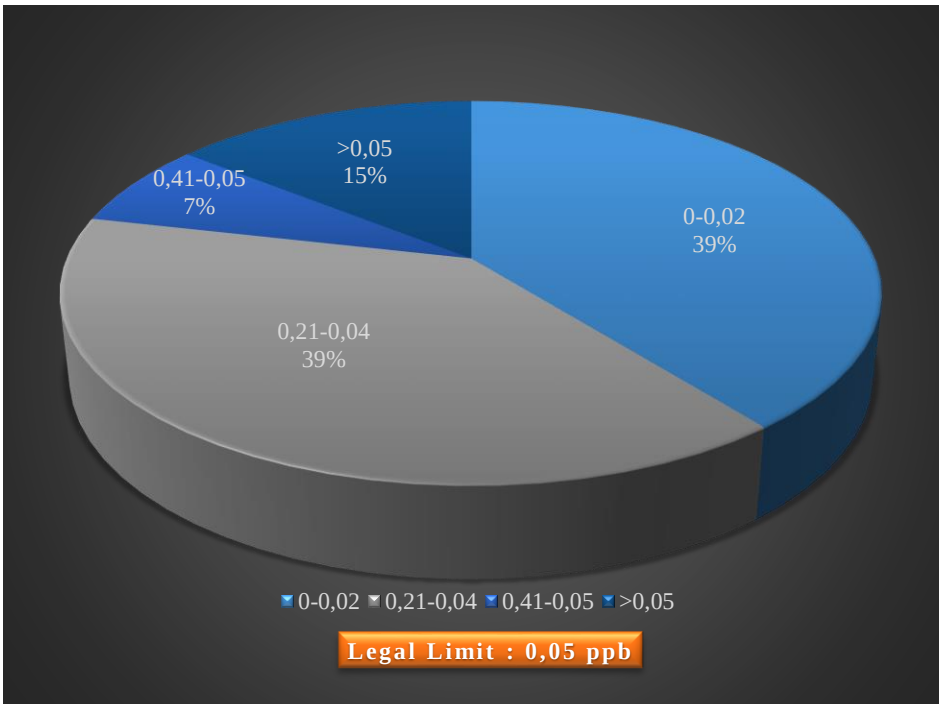

Figure 1. Aflatoxin M_1 Concentration Percentages of Analyzed Raw Milk Samples

3.2. Heavy Metal Results

As a result of heavy metal and mineral substance analysis with ICP-MS; The contents of mean heavy metals in raw milk samples were found that 41.96±1.88 ppm for Mg; 79.83±4.30 ppm for Ca; 0.0115±0.007 ppm for Mn; 0.176±0.003 ppm for Fe; 0.0283±0.002 ppm for Ni; 0.0181±0.003 ppm for Cu; 1.56±0.05 ppm for Zn; 0.0046±0.000 ppm for As; 0.001549±0.002 ppm for Cd and 0.000488±0.000 ppm for Pb (Table 2 and 3).

Table 2. Heavy metal levels (ppm)

	Mg	Ca	Mn	Fe	Ni
Acıgöl	39,63±3,00^{b}	76,28±3,70^{b}	0,01245±0,009^{b}	0,146±0,002^{b}	0,040±0,004^{d}
Avanos	43,21±3,27^{b}	83,79±5,50^{bc}	0,011375±0,009^{b}	0,202±0,009^{c}	0,031±0,005^{bc}
Gülşehir	42,46±5,67^{b}	81,70±3,46^{bc}	0,010875±0,007^{b}	0,163±0,009^{b}	0,024±0,009^{b}
Derinkuyu	31,48±1,13^{a}	60,37±2,73^{a}	0,008375±0,005^{a}	0,130±0,009^{a}	0,021±0,007^{a}
Hacıbektaş	43,79±2,19^{b}	187,20±7,5^{d}	0,0112±0,008^{b}	0,218±0,006^{d}	0,027±0,003^{b}
Kozaklı	42,48±2,02^{b}	79,49±4,57^{b}	0,0114±0,009^{b}	0,178±0,003^{b}	0,034±0,002^{c}
Ürgüp	50,66±2,51^{c}	96,01±4,08^{c}	0,0152±0,010^{c}	0,193±0,000^{c}	0,023±0,005^{b}

For a given metal, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

Tablo 3. Heavy metal levels (ppm)

	Cu	Zn	As	Cd	Pb
Acıgöl	0,0199±0,009^{c}	1,52±0,421^{b}	0,0116±0,011^{d}	0,004465±0,007^{c}	0,0006±0,000^{d}
Avanos	0,0187±0,004^{b}	1,66±0,892^{bc}	0,0057±0,004^{c}	0,001525±0,001^{bc}	0,0005±0,000^{c}
Gülşehir	0,0180±0,003^{b}	1,65±0,714^{bc}	0,0038±0,003^{b}	0,001075±0,001^{b}	0,0003±0,000^{b}
Derinkuyu	0,0134±0,009^{a}	0,95±0,183^{a}	0,0036±0,001^{b}	0,0007±0,000^{a}	0,0003±0,000^{b}
Hacıbektaş	0,0186±0,002^{b}	1,88±1,621^{c}	0,0024±0,001^{b}	0,00135±0,001^{bc}	0,0007±0,000^{d}
Kozaklı	0,0182±0,006^{b}	1,43±0,744^{b}	0,0018±0,001^{a}	0,00095±0,000^{b}	0,0004±0,000^{c}
Ürgüp	0,0199±0,006^{c}	1,80±1,051^{c}	0,0031±0,003^{b}	0,000775±0,000^{ab}	0,0002±0,000^{a}

For a given metal, mean concentrations followed by the same letter are not significantly different ($p<0.05$).

Statistical evaluation of heavy metal results was made using SPSS 15.0 package program. ANOVA test was used for statistical comparison of the means. The statistical significance of the results obtained was evaluated at the level of $p<0.05$ and $p<0.01$ and expressed using different letters in the Tables 2 and 3.

When the results are examined; the lowest values were determined in Derunkuyu district for Mg, Ca, Mn, Fe, Ni, Cu, Zn and Cd elements. The lowest value was determined in Kozaklı district for As. The lowest value was determined in Ürgüp district for Pb.

The highest values were determined in Ürgüp district for Mg, Ca, Mn, Fe, Cu and Zn elements. The highest values were determined in Kozaklı district for Pb and Ni. The highest values were determined in Acıgöl district for As and Cd.

4. DISCUSSION AND CONCLUSION

In a study conducted in 40 long-life milk samples in our country; the mean amount of Aflatoxin M_1 has been reported as 0.0029±0.000 µg/L [13].

Temamoğulları and Kanıcı determined that in Şanlıurfa with the ELISA method; In 38 raw milk samples and 12 UHT milk samples, the average Aflatoxin M1 amounts were determined as 56.74±40.32 ng/kg and 43.1±23.19 respectively.

Hussain and Anwar [15] reported that in 2008, 99.4% of 168 raw milk samples exceeded EU limits for Aflatoxin M1 content.

Almeida Picinin et al. [16] were determined, in three different climatic conditions, with the ELISA method, on 129 raw milk samples in Brazil; They found the mean amount of aflatoxin M_1 to be 0.0195±0.0021 µg/l. They stated that all of the samples were in compliance with the Brazilian legal limit (0.5 µg/L), and 18 samples (13.95%) exceeded the Codex Alimentarius and EU limits (0.05 µg/L).

In the study of Fallah and his colleagues in which they examined 88 raw milk samples in Iran by TLC; They found the amount of aflatoxin M_1 in the range of 0.013-0.394 µg/L and an average of 0.052±0.006 µg/L [17].

Li et al.; They found the average Aflatoxin M_1 amount of 5650 raw milk samples collected from main milk producers in China during 2016 to be 36.8±43.6 ng/L. They stated that only 63 of the samples exceeded the EU limits [18].

In a study by Duarte and his colleagues in pasteurized and UHT milk; They reported the mean amount of aflatoxin M_1 as 23.4±24.0 ng/L [19].

The findings obtained as a result of Aflatoxin M_1 analyzes in this study; It was found higher than the findings of Almeida Picinin et al., Duarte et al.; It is similar to the study of Kabak and Özbey. [13,16,19]. On the other hand, the mean amount of Aflatoxin M_1 was found to be lower than the findings of Fallah et al., Temamogullari and Kanici, Li et al., Hussain and Anwar [14-15,17-18].

In the study, Aflatoxin M_1 was detected in 75% of raw milk samples and 15% were not in compliance with the Turkish Food Codex Contaminants Regulation. It points out the importance of producing, transporting and storing the feed consumed by animals in suitable conditions, and raising the awareness of producers and consumers on these issues.

Heavy metal results obtained in our study and literature data are shown in Table 4.

Table 4. Findings Obtained as a result of Heavy Metal Analysis Performed by ICP-MS in Our Study and Comparison with Literature Data

Heavy metals (ppm)		**National Food Composition Database (ppm) [20]**	**Özturan (ppm) [21]**	**Özrenk (ppm) [22]**	**Birghila et. al. (ppm) [23]**	**Lindmark-Mansson et. al (ppm) [24]**	**Licata et.al. [25]**	**Gövercin [26]**	**İstanbulluoğlu [27]**	**Rana et. al. [28]**	**Çakır [29]**
Mg	41,96±18,88	90	107,33	45,601	214						
Ca	79,83±78,30	980	1258,48	568,104		1140					
Mn	0,0115±0,0079		0,022	0,066	0,08	LOD					
Fe	0,176±0,122	0,2	0,640	0,309	0,72	0,4					
Ni	0,0283±0,028		0,034	0,189	0,04						
Cu	0,0181±0,013		0,079	0,182	0,17	0,1	0,00198				
Zn	1,56±0,85		1,406	3,003	0,98	4,4	2,016				
As	0,0046±0,005						0,0379	<0,003	0,00068	0,156±0,009	
Cd	0,001549±0,0028				0,000004		0,00002		≤0,00016		0,00009±0,00009
Pb	0,000488±0,0004				0,00012		0,00132	<0,01			0,008±0,009

When compared with the literature data, it was observed that the mineral substances obtained in this study were low. It is known that reasons such as breed, species, lactation status, seasonal conditions, poor and malnutrition cause mineral substance changes in milk.

When the heavy metal findings were examined in the study, it was seen that, they were not at a level to risk to human health.

Since milk is a sensitive product; should not be forgotten that pastures where animals are fed, water consumed by animals, various environmental and industrial pollutants, pesticides, veterinary drugs used in animal treatment, detergents used in cleaning and disinfection can contaminate milk.

Control of pollutants that may occur in milk; It is important for both human and public health and milk technology

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CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

REFERENCES

[1] Yetişemiyen A, Süt Üretimi, Süt Hayvancılığı, Sütün Oluşumu ve Sağımı. Süt Teknolojisi. Editör/ Yetişemiyen A, Ankara, 2013.

[2] T.C. Cumhurbaşkanlığı Mevzuat Bilgi Sistemi Türk Gıda Kodeksi Bulaşanlar Yönetmeliği. https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=15692&MevzuatTur=7&MevzuatTertip=5

[3] Blanco JL, Carrioon BA, Liria N, Diaz S, Garcia ME, Dominguez L, Suarez G, Behavior of Aflatoxins During Manifacture and Storage of Yoghurt. Milchwiss, 1993; 48: 385-387.

[4] Badea M, Micheli L, Messia MC, Candigliota T, Marcani E, Mottram T, Velasco-Garcia M, Moscone D, Palleschi G, Aflatoxin M1 Determination in Raw Milk Using a Flow –Injection Immunoassay System. Anal Chim Acta, 2004; 520: 141-148.

[5] Vural H, Ağır Metal İyonlarının Gıdalarda Oluşturduğu Kirlilikler. Çevre Dergisi, 1993; 8: 3-8.

[6] Sağlam N, Cihangir N, Ağır Metallerin Biyolojik Süreçlerle Biyosorbsiyonu Çalışmaları. Hacettepe Üniversitesi Eğitim Fakültesi Dergisi, 1995; 11: 157-161.

[7] Kahvecioğlu Ö, Kartal G, Güven A, Timur S, Metallerin Çevresel Etkileri-I. Metalurji Dergisi, 2004; 136: 47-53.

[8] Metin M, Süt Teknolojisi. Sütün Bileşimi ve İşlenmesi. Ege Üniversitesi Basımevi:Yayın No:33, İzmir, 2001.

[9] TS EN ISO 14501, Süt ve Süt Tozu-Aflatoksin M1 Muhtevası Tayini-İmmunoaffiniti Kromatografi ile Temizleme ve Yüksek Performanslı Sıvı Kromatografi ile Tayini. 2008.

[10] Committee on Food Analysis, Trace elements, As, Cd, Hg, Pb and other elements. Determination by ICP-MS after pressure digestion. No:186, 14, 2007.

[11] Li S, Min L, Wang P, Zhang Y, Zheng N, Aflatoksin M1 Contamination in Raw Milk from Major Milk-Producing Areas of China during Four Seasons of 2016. Food Control, 2017; 82: 121-125.

[12] An Official Website of the European Union. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32006R1881

[13] Kabak B, Özbey F, Aflatoxin M1 in UHT Milk in Consumed in Turkey and First Assessment of its Bioaccessibility Using an in Vitro Gestion Model. Food Control, 2012; 28: 338-344.

[14] Temamoğulları F, Kanıcı A, Short Communication: Aflatoxin M1 in Dairy Products Sold in Şanlıurfa, Turkey. J Dairy Sci, 2014; 97: 162-165.

[15] Hussain I, Anwar J, A Study on Contamination of Aflatoxin M1 in Raw Milk in the Punjab Province of Pakistan. Food Control, 2008; 19: 393-395.

[16] Almeida Picinin LC, Oliveira Pinho Cerqueira MM, Vargas EA, Quintão Lana AM, Maia Toaldo I, Bordignon-Luiz M., Influence of Climate Conditions on Aflatoxin M1 Contamination in Raw Milk From Minas Gerais State, Brazil. Food Control, 2013; 31: 419-424.

[17] Fallah AA, Rahnama M, Jafari T, Siavash Saei-Dehkordi S, Seasonal Variation of Aflatoxin M1 Contamination in Industrial and Traditional Iranian Dairy Products. Food Control, 2011; 22: 1653-1656.

[18] Li S, Min L, Wang P, Zhang Y, Zheng N, Aflatoksin M1 Contamination in Raw Milk from Major Milk-Producing Areas of China during Four Seasons of 2016. Food Control, 2017; 82: 121-125.

[19] Duarte SC, Almeida AM, Teixeira AS, Pereira AL, Falcão AC, Pena A, Lino CM, Aflatoxin M1 in Marketed Milk in Portugal: Assessment of Human and Animal Exposure. Food Control, 2013; 30: 411-417.

[20] T.C. Tarım ve Orman Bakanlığı, Ulusal Gıda Kompozisyon Veri Tabanı. http://www.turkomp.gov.tr/food-17

[21] Özturan K, Erzurum ve Çevresinde Üretilen Süt ve Süt Ürünlerinin Mevsimlere Göre Mineral Madde ve Ağır Metal İçeriği. Atatürk Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, Erzurum, 2010.

[22] Özrenk Ö, Van İli ve İlçelerinde Üretilen İnek Sütlerinin Ağır Metal Kirlilik Düzeyi ve Bazı Mineral Madde İçerikleri. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, Van, 2002.

[23] Birghila S, Dobrinas S, Stanciu G, Soceanu A, Determinatipon of Major and Mınor Elements in Milk Through ICP-AES. Environ Eng Manag J, 2008; 7: 805-808.

[24] Lindmark-Mansson H, Fonden R, Pettersson HE, Composition of Swedish Dairy Milk. Int Dairy J, 2003; 13: 409-425.

[25] Licata P, Trombetta D, Cristani M, Giofre F, Martino D, Calo M, Naccari F, Levels of Toxic and Essential Metals in Samples of Bovine Milk From Various Dairy Farms in Calabria, Italy. Environ Int, 2004; 30: 1-6.

[26] Gövercin İ, İzmir İlinde Sütlerde Bazı Ağır Metal (Kurşun, Kadmiyum, Arsenik, Civa, Bakır, Çinko) Düzeylerinin Belirlenmesi. Ege Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, İzmir, 2010.

[27] İstanbulluoğlu H, Piyasada Satılan Süt ve Süt Ürünlerinde Ağır Metal Kirliliği. GATA Halk Sağlığı Anabilim Dalı Başkanlığı, Tıpta Uzmanlık Tezi, Ankara, 2011.

[28] Rana T, Sarkar S, Mandal T, Bhattacharyya K, Roy A, Kol L, Contribution of Arsenic from Agricultural Food Chain to Cow Milk in Highly Arsenic Prone Zone in Nadia District of West Bengal in India. Internet J Veterin Med, 2007; 4: 1-6.

[29] Çakır EO, Türkiye'nin Farklı Bölgelerinden Toplanan Süt Örneklerinde Bazı Metal Düzeyleri. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü Doktora Tezi, Ankara, 2009.